

Association of Human-Leukocyte-Antigen Class I (B*0703) and Class II (DRB1*0301) Genotypes with Susceptibility and Resistance to the Development of Severe Acute Respiratory Syndrome

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Severe acute respiratory syndrome (SARS) is a public health concern worldwide. By studying the human leukocyte antigen (HLA) types A, B, DR, and DQ alleles in 90 Chinese patients with serologically confirmed SARS infections, we identified a strong association between HLA-B*0703 (OR, 4.08; 95% CI, 2.03–8.18; $P = .00072$ [Bonferroni-corrected P value, $P_c < .0022$]) and -DRB1*0301 (OR, 0.06; 95% CI, 0.01–0.47; $P = .00008$ [after Bonferroni correction, $P < .0042$]) and the development of SARS. Moreover, the frequency of B*0703 and B60 coinheritance (9.6%; 95% CI, 4.6%–19.0%) in our SARS group was significantly higher ($P = 3 \times 10^{-9}$) than that expected in the general population (0.4%). These genetic data will critically affect both the study of the pathogenesis of SARS and the design of vaccination programs.

Severe acute respiratory syndrome (SARS) is a newly described human infectious disease caused by a novel coronavirus, SARS-CoV [1, 2]. Since its first appearance, in November 2002, $> \geq 8,000$ people have been infected, in multiple outbreaks globally. SARS infection is important because of its high infectivity and unpredictable clinical course, which is characterized by a

high mortality rate [1]. It has been observed that clinical severity in patients may vary, from a febrile condition with mild respiratory symptoms and radiological evidence of lung involvements to severe respiratory distress with extensive lung damage requiring assisted ventilation [1, 3]. Individual susceptibility to infection by SARS-CoV also appear to be variable. Lymphopenia (absolute lymphocyte count, $< 1000/\text{mm}^3$) is commonly observed with the onset of clinical symptoms. T lymphocytes seem preferentially destroyed, but B cells are spared [1, 4]. Although it is evident that lung involvement is immunological in nature, there is thus far no known effective treatment of this infection [1, 3]. In most of autoimmune pathogenesis, both environmental and genetic factors—especially HLA genotypes, which influence the ability of T cells to respond to a particular antigen or viral protein—clearly are important [5]. There is a strong association between infection and the onset of autoimmunity, which suggests that infectious agents play a critical role in the process.

Patients and methods. To assess the association between HLA genotypes with susceptibilities or resistance to SARS infection and disease severity as indicated by admissions to intensive care units (ICUs) or deaths, we recruited, for HLA genotyping, 90 serologically confirmed patients who fulfilled the World Health Association (WHO) case definition of SARS [6] and who were being managed in multiple hospitals in Hong Kong between March and June 2003. Informed consent was obtained from the patients or their guardians, according to institutional guidelines. All the patients with SARS whom we included in the study were genetically unrelated Chinese; the male:female ratio was 1:1.1, and the age range was 22–85 years. Of the 90 patients studied, 18 had a history of admission to ICUs, and 7 died during the SARS episode. Archival peripheral blood (PB) samples collected in EDTA bottles and remaining after routine blood tests were used; inadequate materials were obtained from 3 samples for HLA-A analysis, 7 samples for HLA-B analysis, and 11 samples for HLA-DR analysis.

Genomic DNA was isolated from patients' PB samples by standard phenol/chloroform/isoamyl alcohol extraction and ethanol precipitation. The quantity and quality were assessed by spectroscopic absorbance at 260 and 280 nm. The HLA class I and class II alleles were genotyped by polymerase chain reaction using sequence-specific primers, as described elsewhere [7].

The frequencies of HLA alleles found in our patients with SARS were then compared with published frequencies of these alleles in a population of unrelated Hong Kong Chinese bone-marrow donors (class I—allele group, $N = 18,774$; class II—allele

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group, $N = 250$) taken from the Hong Kong Marrow Match Registry [8]; this registry comprises individuals with an age range of 18–55 years, and a male:female ratio slightly <1 (i.e., it includes slightly more women than men), as is true of the overall Hong Kong population (B. Hawkins, personal communication). Hong Kong is located south of China. More than 98% of the population are Chinese, and the majority have ancestral origins in Guangdong Province in southern China and are of the Han ethnic group [8]. In terms of age, sex, and ethnic group, our patients with SARS had a stratification similar to that of this control population, which is thus far the most reliable representation of Hong Kong Chinese.

Although in the previous study the typing of HLA-A and -B in Hong Kong Chinese bone-marrow donors was performed by standard serology techniques [8], the serology typing results have been demonstrated to have high concordance with the results of DNA typing [9]; with regard to HLA-B*0703 or -B60 typing, in particular, no discrepancy between serological and DNA-based data has been found [9]. The odds ratio (OR) and respective 95% confidence interval (CI) were calculated by 2-way contingency-table analysis, to determine the magnitude of association between HLA alleles and SARS infection [10]. To assess significance, Fisher's exact test was used to further compare the allele frequencies found in our group of patients with SARS versus the allele frequencies in the control group; a 2-tailed $P < .05$ was considered to be statistically significant [10]. For multiple comparisons, the significance level (P_c) was adjusted by dividing the number of alleles detected at each locus, according to Bonferroni correction [11].

Results and discussion. The results of Fisher's exact test showed that, in our patients with SARS, the frequencies of HLA-B*0703, -B67, -DRB1*0901, and -DQB1*0601 were significantly higher, and the frequencies of HLA-B75, -DRB1*0301, and -DQ7*DQB1*0301 significantly lower, than those in the overall Hong Kong Chinese population (table 1). After Bonferroni correction, frequencies of HLA-B*0703 (OR, 4.08 [95% CI, 2.03–8.18]; $P = .00072$ [after Bonferroni correction, $P < .0022$]) and -DRB1*0301 (OR, 0.06 [95% CI, 0.01–0.47]; $P = .00008$ [after Bonferroni correction, $P < .0042$]) in our patients with SARS remained statistically significantly different than those in the overall Hong Kong Chinese population (table 1), suggests the presence of an underlying genetic susceptibility and resistance—conferred by the HLA-B*0703 and the HLA-DRB1*0301 polymorphisms, respectively—with regard to the development of SARS. Except for a trend toward a higher frequency of HLA-B13 in the ICU-admitted patients with SARS versus the non-ICU-admitted patients with SARS ($P = .06$), there was no correlation between HLA genotypes and either ICU admissions or deaths related to SARS. A previous HLA study, conducted in a group of 33 Taiwanese patients who were probably infected with SARS, suggested that the fre-

quency of the HLA-B*4601 genotype was significantly higher in a subgroup of 6 patients with severe SARS infections than in a group of 101 high-risk uninfected health-care workers [12]. In our study, however, we did not observe any association between HLA-B*4601 and SARS; even in the 16 ICU-admitted patients with SARS (with known HLA-B typing) and in the 7 patients with SARS who died during the episode, the frequency of HLA-B*4601 was not significantly higher than that in the overall Hong Kong Chinese population.

Of special interest is the strong association, in our patients with SARS, between HLA-B*0703 and -B60: 8 of the 9 patients with HLA-B*0703 coinherited HLA-B60, which alone did not demonstrate any excessive risk of SARS. The frequency of B*0703 and B60 coinheritance (9.6% [95% CI, 4.6%–19.0%] in our patients with SARS was significantly higher than the frequency expected (0.4%) in the overall Hong Kong Chinese population ($P = 3 \times 10^{-9}$). It is possible that B*0703 confers a genetic susceptibility to infection with SARS, whereas B60 may play a secondary role in the pathogenesis. On the other hand, our data support the idea that DRB1*0301 provides protection against infection with SARS. It will be worthwhile to conduct further biological and functional studies involving specific HLA proteins found in the present study, to clarify their participation in the pathogenesis of SARS. Although the involvement of these HLA proteins in the pathogenesis of SARS remains to be explored, the understanding of the genetic risks or protection conferred can be viewed from 2 perspectives: in relation to (1) the control of viral proliferation and persistence and (2) the participation in autoimmune pathogenesis. In the former perspective, studies of hepatitis C virus (HCV) and infection with human papillomavirus suggest that certain HLA genotypes influence the host's ability to clear the virus and avoid persistence [13–15]. There is evidence that, in HCV infection, DQB1*0301 provides a protective effect by linking with the antiviral-effector pathway involving CD4 helper cells [13]. Genetic-association studies have strongly suggested that class II restricted responses may be important in immunological control of disease outcome, although the genes implicated in different studies vary [13]. Ninety-eight percent of our patients with SARS developed lymphopenia, which characteristically marks the onset of symptoms. Decreases of both CD4⁺ and CD8⁺ cells were also commonly observed and, when found at presentation, were associated with adverse outcomes [4]. By contrast, there was a relatively stable preservation of B cells [4]. It can be postulated that the consistent early drop in the CD4 counts observed in patients with SARS [4] may be evidence that impairment of this CD4 T cell response affects SARS-CoV proliferation in DRB1*0301–negative patients with SARS.

Alternatively, there is a link between these HLA loci and other unknown determinants that govern both the course of infection with SARS and subsequent lung damage. In addition

Table 1. Frequency of HLA A, B, DR, and DQ alleles in Hong Kong Chinese patients with SARS and in the overall Hong Kong Chinese population.

Class I allele	Patients with SARS, no. (%) of sample	Hong Kong Chinese, no. (%) of sample	OR (95% CI)	<i>P</i> ^a
HLA-A (patients with SARS, <i>n</i> = 87; HK Chinese sample, <i>n</i> = 18,774; after Bonferroni correction, <i>P</i> < .0045)				
A2	49 (56.3)	10,001 (53.3)	1.13 (0.74–1.73)	.5918
A3	1 (1.1)	414 (2.2)	0.52 (0.07–3.71)	1.0000
A11	46 (52.9)	10,557 (56.2)	0.87 (0.57–1.33)	.5884
A24	21 (24.1)	5,047 (26.9)	0.87 (0.53–1.42)	.6288
A25	1 (1.1)	21 (0.1)	10.38 (1.38–78.06)	.0968
A*2601	2 (2.3)	743 (4.0)	0.57 (0.14–2.33)	.5868
A29	4 (4.6)	459 (2.4)	1.92 (0.70–5.27)	.1657
A30	3 (3.4)	586 (3.1)	1.11 (0.35–3.52)	.7537
A31	1 (1.1)	548 (2.9)	0.32 (0.05–2.33)	.5231
A33	15 (17.2)	3528 (18.8)	0.90 (0.52–1.57)	.7846
A*3402	1 (1.1)	22 (0.1)	9.91 (1.32–74.36)	.1009
HLA-B (patients with SARS, <i>n</i> = 83; HK Chinese sample, <i>n</i> = 18,774); after Bonferroni correction, <i>P</i> < .0022				
B13	20 (24.1)	3298 (17.6)	1.49 (0.90–2.47)	.1466
B18	1 (1.2)	149 (0.8)	1.52 (0.21–11.02)	.4854
B*2706,4	2 (2.4)	769 (4.1)	0.58 (0.14–2.36)	.7770
B35	3 (3.6)	1163 (6.2)	0.57 (0.18–1.80)	.4901
B37	1 (1.2)	180 (1.0)	1.26 (0.17–9.10)	.5517
B38	8 (9.6)	1866 (9.9)	0.97 (0.47–2.01)	1.0000
B39	3 (3.6)	514 (2.7)	1.33 (0.42–4.23)	.4977
B45	1 (1.2)	85 (0.5)	2.68 (0.37–19.49)	.3163
B46	27 (32.5)	5125 (27.3)	1.28 (0.81–2.04)	.3229
B*4801	2 (2.4)	626 (3.3)	0.72 (0.18–2.92)	1.0000
B51	6 (7.2)	1726 (9.2)	0.77 (0.34–1.77)	.7027
B52	1 (1.2)	213 (1.1)	1.06 (0.15–7.67)	.6130
B53	1 (1.2)	103 (0.5)	2.21 (0.31–16.03)	.3687
B54	5 (6.0)	782 (4.2)	1.48 (0.60–3.65)	.3989
B55	2 (2.4)	788 (4.2)	0.56 (0.14–2.30)	.5864
B*5801	15 (18.1)	2416 (12.9)	1.49 (0.85–2.62)	.1860
B60	21 (25.3)	5172 (27.5)	0.89 (0.54–1.46)	.7129
B61	3 (3.6)	997 (5.3)	0.67 (0.21–2.12)	.8033
B62	13 (15.7)	1769 (9.4)	1.79 (0.99–3.23)	.0594
B14/B64/B65	1 (1.2)	12 (0.1)	19.07 (2.45–148.34)	.0558
B67	3 (3.6)	81 (0.4)	8.65 (2.68–27.97)	.0061
B*0703	9 (10.8)	544 (2.9)	4.08 (2.03–8.18)	.00072
B75	7 (8.4)	3552 (18.9)	0.40 (0.18–0.86)	.0112
HLA-DRB (patients with SARS, <i>n</i> = 79; HK Chinese sample, <i>n</i> = 250; after Bonferroni correction, <i>P</i> < .0042)				
DRB1*04	16 (20.3)	49 (19.6)	1.04 (0.55–1.96)	.8705
DRB1*0301	1 (1.3)	42 (16.8)	0.06 (0.01–0.47)	.00008
DRB1*07	4 (5.1)	26 (10.4)	0.46 (0.16–1.36)	.1825
DRB1*08 (not 0809)	7 (8.9)	19 (7.6)	1.18 (0.48–2.93)	.8109
DRB1*0901	33 (41.8)	73 (29.2)	1.74 (1.03–2.94)	.0395
DRB1*1001	5 (6.3)	11 (4.4)	1.47 (0.49–4.36)	.5484
DRB1*11	10 (12.7)	23 (9.2)	1.43 (0.65–3.15)	.3921
DRB1*12	19 (24.1)	83 (33.2)	0.64 (0.36–1.14)	.1625
DRB1*13	2 (2.5)	13 (5.2)	0.47 (0.11–2.15)	.5358
DRB1*14	13 (16.5)	36 (14.4)	1.17 (0.59–2.34)	.7171
DRB1*15	17 (21.5)	60 (24.0)	0.87 (0.47–1.60)	.7608
DRB1*16	7 (8.9)	30 (12.0)	0.71 (0.30–1.69)	.5426
HLA-DQ (patients with SARS, <i>n</i> = 90; HK Chinese sample, <i>n</i> = 250; after Bonferroni correction, <i>P</i> < .0063)				
DQB1*02	16 (17.8)	63 (25.2)	0.64 (0.35–1.18)	.1901
DQB1*04	13 (14.4)	28 (11.2)	1.34 (0.66–2.72)	.4513
DQB1*05	29 (32.2)	95 (38.0)	0.78 (0.47–1.29)	.8655
DQB1*0601	27 (30.0)	42 (16.8)	2.12 (1.21–3.71)	.0095
DQB1*0603–9	3 (3.3)	6 (2.4)	1.40 (0.34–5.73)	.7037
DQ7/DQB1*0301	25 (27.8)	100 (40.0)	0.58 (0.34–0.98)	.042
DQ8/DQB1*0302	12 (13.3)	24 (9.6)	1.45 (0.69–3.03)	.3231
DQ9/DQB1*0303	31 (34.4)	75 (30.0)	1.23 (0.74–2.05)	.4299

NOTE. CI, confidence interval; HK, Hong Kong; OR, odds ratio; SARS, severe acute respiratory syndrome.

^a Uncorrected 2-tailed value, by Fisher's exact test.

to these implicated genes, other closely linked genes in the HLA class II region, such as TNF- α , may also play important roles. To gain additional insight into the roles that these HLA polymorphisms play in susceptibility and resistance to SARS, we will perform further HLA testing the families of patients with SARS, considering genetically related and unrelated family members who have been affected or unaffected after close household exposure to the SARS index patients. The identification of individuals at risk for infection with SARS may help in the selection of priority candidates for vaccination against influenza at this stage; and, in the future, once the SARS vaccine is successfully developed, it may help in the selection of candidates for vaccination against SARS. Meanwhile, we would advise that health-care workers who harbor HLA-susceptible genotypes be considered for exemption from interaction with patients infected with SARS.

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